1: J Biotechnol. 1999 Feb 19;68(2-3):101-13. Related Articles, L

Cloning, nucleotide sequence and expression of a new L-N-carbamoylase gene from Arthrobacter aurescens DSM 3747 in E. coli.

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An L-N-carbamoyl amino acid amidohydrolase (L-N-carbamoylase) from Arthrobacter aurescens DSM 3747 was cloned in E. coli and the nucleotide sequence was determined. After expression of the gene in E. coli the enzyme was purified to homogeneity and characterized. The enzyme was shown to be strictly L-specific and exhibited the highest activity in the hydrolysis of beta-aryl substituted N alpha-carbamoyl-alanines as e.g. N-carbamoyl-tryptophan. Carbamoyl derivatives of beta-alanine and charged aliphatic amin acids were not accepted as substrates. The N-carbamoylase of A. aurescens DSM 3747 differs from all known enzymes with respect to its substrate specificity although amino a sequence identity scores of 35-38% to other N-carbamoylases have been detected. The enzyme consists of two subunits of 44,000 Da, and has an isoelectric point of 4.3. The optima of temperature and pH were determined to be 50 degrees C and pH 8.5 respective At 37 degrees C the enzyme was completely stable for several days.

PMID: 10194852 [PubMed - indexed for MEDLINE]

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1: FEMS Microbiol Lett. 1996 Nov 15;145(1):55-62.

ELSEVIER
FULL-TEXT ARTICLE

Identification, sequencing and mutagenesis of the gene for a D-carbamoylase from Agrobacterium radiobacter.

Buson A, Negro A, Grassato L, Tagliaro M, Basaglia M, Grandi C, Fontana A, Nut MP.

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A clone positive for D-carbamoylase activity (2.7 kb HindIII-BamHI DNA fragment) wa obtained by screening a genomic library of Agrobacterium radiobacter in Escherichia co This DNA fragment contains an open reading frame of 912 bp which is predicted to enco a peptide of 304 amino acids with a calculated molecular mass of 34247 Da. The D-carbamoylase gene, named cauA, was placed under the control of T7 RNA-dependent promoter and expressed in E. coli BL21(DE3). After induction with isopropyl-thio-beta-galactopyranoside, the synthesis of D-carbamoylase in E. coli reached about 40% of the total protein. The expressed protein was shown to possess a molecular mass, on SDS-PAGE, of 36 kDa and showed an enhanced stability with respect to that of the wild-type enzyme derived from A. radiobacter. Site-directed mutagenesis experiments allowed us establish that a Pro14-->Leu14 exchange leads to an inactive enzyme species, while a Cys279-->Ser279 exchange did not impair the functional properties of the enzyme.

PMID: 8931327 [PubMed - indexed for MEDLINE]

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